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Selective saccharification of microwave-assisted glycerol pretreated corncobs via fast pyrolysis and enzymatic hydrolysis

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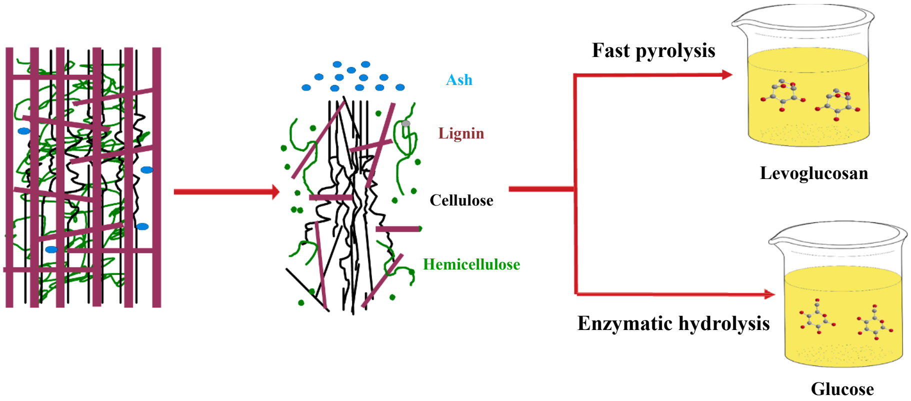
GRAPHICAL ABSTRACT



Fermentable sugars production from microwave-assisted glycerol pretreated biomass

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ABSTRACT

Fermentable sugars are a group of key intermediate compounds and pretreatment is the most crucial step for the saccharification of lignocellulose. In this manuscript, microwave-assisted glycerol pretreatment was performed before fast pyrolysis and enzymatic hydrolysis to obtain fermentable sugars from corncobs. Owing to demineralization, delignification, and concentration of crystalline cellulose via pretreatment, the yield of levoglucosan (44.5%) was significantly increased as compared to that of un-pretreated corncobs (6.9%) in fast pyrolysis. Furthermore, the microwave-assisted glycerol pretreatment also facilitated glucose production in enzymatic hydrolysis. The yield of glucose increased from 19.9 to 52.1%, which could be ascribed to the elimination of lignin by pretreatment. These results demonstrated that microwave-assisted glycerol pretreatment was an economically attractive route to boost the final production of fermentable sugars from lignocellulose via following fast pyrolysis and enzymatic hydrolysis.

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1. Introduction

Two growing global contradictions: the contradiction between growing demands for energy and limitations on fossil energy; the contradiction between human's inclination for high living quality and increased content of environmental pollution drive the development of more sustainable energy systems [1,2]. Fermentable sugars, which can be converted to a variety of biofuels or value-added products, are considered as one of the important intermediate products in the utilization of lignocellulose. The development of lignocellulosic saccharification has attracted global attention all over the world.

Historically, research effort has focused on biochemical or thermochemical processes for lignocellulose saccharification. A typical biochemical process consists of pretreatment to overcome the resistance of lignocellulose, followed by enzymatic hydrolysis to degrade polysaccharide into monosaccharide (mainly glucose) [3]. Fast pyrolysis is a little-explored thermochemical process to obtain fermentable anhydrosugars, which is composed of pretreatment to change both the component and structure of lignocellulose, followed by decomposition at a high temperature (about 500 °C) with a short residence time (less than 2 s) in the absence of oxygen. In fast pyrolysis of cellulose, levoglucosan is the major product and its yield is achieved as high as 60% [4,5]. Levoglucosan can be assimilated by lots of eukaryotic and prokaryotic microorganisms aiming at the production of itaconic acid, lipid and ethanol, giving similar yields and rates as glucose fermentation [6]. Levoglucosan also can be easily hydrolyzed into glucose under the catalysis of mild acid, thereby providing an alternative route to biorefinery. Meanwhile, from the perspective of economy, fast pyrolysis is comparable commercially with enzymatic hydrolysis. Therefore, levoglucosan obtained from fast pyrolysis of lignocellulose, has a huge potential to act as a promising fermentative substrate to produce biofuels and other platform chemicals. Nevertheless, the levoglucosan yield from fast pyrolysis of lignocellulose is commonly evaluated as low that it causes the downstream utilization practically difficult. Obtaining high yields of fermentable sugar from lignocellulose is considered as a vital bottle-neck for the valorization of both enzymatic hydrolysis and fast pyrolysis.

Pretreatment prior to enzymatic hydrolysis or fast pyrolysis is one of the most universal methods to improve the yields of glucose and levoglucosan [7]. The methods of pretreatment include four categories: (1) physical pretreatments (*e.g.,* high energy radiation, grinding, milling, ect.), (2) chemical pretreatments (*e.g.,* dilute acid or alkalis boiling, ozone oxidation, ect.) [8,9], (3) physiochemical pretreatments (*e.g.,* stem explosion, hydrothermal and organosolv methods) and (4) biological pretreatments [1]. In terms of enzymatic hydrolysis, the objective of pretreatment is to enhance the accessibility of cellulase to cellulose for efficient hydrolysis. This can be achieved by removing lignin and hemicellulose in natural feedstock, destroying crystallinity structure, abating the degree of polymerization, and increasing the surface area of the raw material for facilitating the penetration with enzymes. While, in the case of fast pyrolysis, the aim of pretreatment is mainly to demineralize and remove lignin mean to maximal production of levoglucosan from cellulose [10]. The ash, especially alkali and alkaline earth metals (AAEMs) in lignocellulose, has been investigated to possess a catalytic effect to negatively suppress the primary depolymerization reactions responsible for yielding levoglucosan [11]. The presence of lignin promotes the fast pyrolysis of cellulose to form low molecular weight products rather than levoglucosan [12]. Since the primary cost element in production of bio-ethanol is pretreatment, accounting for approximately 20–40% of the cost of final product. Further researches are necessarily expected to develop both profitable and efficacious pretreatment methods for saccharification of lignocellulose.

In general, factors hindering the large-scale processing of pretreatment are considered as the cost of reactor capital, the energy consumption and the price of chemicals used in pretreatment. Glycerol is a major by-product derived from the biodiesel manufacturing process, which makes up nearly 10 wt.% of biodiesel [13]. The rapid development in biodiesel production makes the glycerol market far saturated and results in a lower price of glycerol (10 cents per pound). Conversely, the excess of glycerol has caused environmental problem and wasted depletion of resources. The comprehensive application of glycerol can relieve the cost of chemicals consumed during the process of pretreatment. High boiling point (290 °C) of glycerol allows this pretreatment performed under atmospheric pressure, thereby high-pressure rectors are not essential in glycerol pretreatment which lower the cost of reactor capital. Furthermore, glycerol can be utilized by microorganisms as feedstock and it is not an inhibition factor even in some cases that some glycerol remains in the enzymatic hydrolysate [14]. Previous research also reported that glycerol pretreatment heated by oil bath had the ability to improve levoglucosan yield (from 2.2 to 35.8%) in fast pyrolysis [15]. However, the energy consumption required for glycerol pretreatment was uncommercial to be industrialize due to the high temperature (240 °C) and long time (3 h). The conduction/convection heating is superficial heat transfer, while the heat is generated and gathered within a small-scale range in the biomass by microwave-assisted heating, thereby rapid heating occurs efficiently. The electromagnetic field generated by microwave can directly interact with biomass to produce heat, which provides great advantages such as energy efficiency for rapid and selective heating, and meanwhile is evaluated environmentally benign [16]. This heating method can also promote physical, chemical or biological reactions in biomass by applying an electromagnetic field to the molecular structure of the heated biomass [17]. Limited literature had reported on the comparison for fast pyrolysis and enzymatic hydrolysis. It was critical to recognize the advantages and disadvantages when choosing available raw materials and technologies. In this research, the glycerol pretreatment assisted with microwave was adopted prior to enzymatic hydrolysis and fast pyrolysis of corncobs to obtain glucose and levoglucosan, respectively. The effect of pretreatment on the alterations of lignocellulosic composition and structure, combined with yields of sugar were illustrated. Ultimately, a comparative study on fast pyrolysis with enzymatic saccharification of lignocellulose was investigated.

2. Experimental

2.1. Materials

Corncobs were bought from Tianjin, China. The sample was cut into scraps (60–80 meshes), and dried at 105 °C for 24 h. Glycerol (AR) was bought from Tianjin Yongda Reagent Co., Ltd (China). The standard chemicals, such as glucose, xylose and levoglucosan were purchased from Sigma (Shanghai).

2.2. Elemental and compositional analysis

A Vario EL cube analyzer (Elementar, Germany) was used to analyze the organic elemental contents (C, H, N) in corncobs. For a typical elemental analysis, about 3 mg corncobs were weighted and wrapped in a dedicated tin boat and then placed in the sample tray. The analysis of AAEMs was accomplished in an inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer, USA). The sample was placed in a cuvette, mixed with 3 mL HNO_3 and 1 mL $HClO_4$ for digestion before ICP-OES analysis. The main components (cellulose, hemicellulose and lignin) of biomass were detected according to the method of National Renewable Energy Laboratory (NREL) [18]. The content of hemicellulose and cellulose contained in corncobs was hydrolyzed to monosaccharides after acid hydrolysis. The sugars were separated by an Aminex HPX-87P column (Bio-Rad) and detected by a refractive index detector in a high performance liquid chromatography (HPLC) system (Alliance 2695, Waters, America). The mobile phase was deionized water at a flow rate of 0.4 mL/min. The contents of sugars were calculated as the fractions of cellulose and hemicellulose. The acid soluble lignin and the acid insoluble lignin were detected by an UV spectrometer and the difference of weight loss, respectively.

2.3. Structural characterization

Thermal stability of biomass was measured by TGA Q50 thermogravimetric analyzer (TA, USA). Sample (about 6–8 mg) was heated from 25 to 700 °C at a rate of 5 °C/min. The furnace was maintained at the final temperature for 0.5 h. To maintain an inert atmosphere, purified nitrogen gas (40 mL/min) was utilized as the purge gas. The X-ray diffraction (XRD) pattern of biomass was obtained on an X-ray diffractometer (PANalytical V.B., Holland) equipped with Cu radiation ($\lambda = 1.54$ Å). Tube voltage and tube current of X-ray diffractometer were set to 40 kV and 40 mA, respectively. The biomass was scanned from 5 to 45°. The crystallinity index (CrI) was calculated as the following equation [19]:

$$\mathrm{CrI}(\%) = \frac{I_{Cr} - I_{AM}}{I_{Cr}} \times 100\% \tag{1}$$

where I_{Cr} denoted the intensity peak for crystalline and amorphous cellulose, and I_{AM} was the intensity peak of amorphous cellulose.

2.4. Microwave pretreatment

The glycerol pretreatment of biomass was conducted in a MCR-3 microwave chemical reactor (Star Shuo Instrument Co., Ltd, China) equipped with microcomputer and thermocouple. Before the pretreatment, biomass and glycerol were mixed with a 10% solid-to-liquid mass ratio and loaded in a 250 mL flat bottom two-neck flask. The pretreatment was performed at 200–240 °C for 1–10 min. 200-1, 200-5, 200-10 denoted the pretreatment carried out at 200 °C for 1, 5 and 10 min, respectively. When the pretreatment was ended, the pretreated corncobs were recovered by filtration, washed thoroughly and then dried in a freeze dryer.

2.5. Fast pyrolysis

Fast pyrolysis experiment was performed in a semi-batch CDS reactor (Pyroprobe 5200, U.S.A.). The compounds were separated and identified by gas chromatograph (Agilent 7890, Agilent Technologies) and mass spectrometer (Agilent 7975C, Agilent Technologies) coupled to the reactor. In each typical run, biomass (about 0.2 mg) was placed in the center of quartz tubes, with quartz wool blocking the terminals of the tube. The biomass was decomposed at 500 °C for 20 s at a heating rate of 20 K ms^{-1}. The pyrolysis compounds were purged from the pyrolyzer to the GC injector by helium (20 mL/min) through transmission line. The method for products separation and detection was reported in previous literature [20]. External standard method was employed for quantitative analysis of levoglucosan. The formula for calculating the yield of levoglucosan and the relative content of other compounds were defined as:

$$\text{Levoglucosan yield (wt. \%)} = \frac{\text{mass of levoglucosan}}{\text{mass of cellulose}} \times 100\% \tag{2}$$

$$\text{Relative content of compound (\%)} = \frac{\text{Area of a compound}}{\text{Area of all compunds}} \times 100\% \tag{3}$$

2.6. Enzymatic hydrolysis

Biomass (0.15 g), sodium citratereaction buffer (50 mM, pH 4.8, 9.8 mL), 2% sodium azide (100 μL), and cellulase (Celluclast 1.5L®, Sigma) equal to 30 FPU/g biomass were added into a 50-mL Erlenmeyer flask for enzymatic saccharification. The activity of enzyme was measured according to the method of NREL [21]. The enzymatic hydrolysis was performed at 50 °C with a shaking speed of 80 rpm. Qualitative and quantitative detection of glucose was obtained on a HPLC system. All experiments were repeated three times. Glucose yield was defined as:

$$\text{Glucose yield } (wt.\ \%) = \frac{\text{mass of glucose}}{\text{mass of cellulose}} \times 0.9 \times 100\% \tag{4}$$

3. Results and discussion

3.1. Elemental analysis

The analysis of organic elements is shown in Table 1. Compared to the raw sample, microwave-assisted glycerol pretreated corncobs showed lower C and N contents, conversely higher H contents. According to the result of ICP-OES, the total content of AAEMs in corncobs drastically declined after microwave-assisted glycerol pretreatment. As for raw material, the sum content of AAEMs was detected as high as 12988.1 ppm. After glycerol pretreatment at 200 °C for 1 min, the content of AAEMs in corncobs reduced by 61.6%. Noteworthy, the content of K was decreased from 7957.5 to 1032.0 ppm. Besides, higher temperatures or longer residence times favored the removal of AAEMs. After pretreatment at 240 °C for 10 min, 32.0% Ca, 48.8% Mg, 35.7% Na and 93.2% K were removed from raw material. AAEMs could alter the electronic structure of cellulose, resulting in promotion of dehydration, rearrangement and fragmentation [13]. AAEMs also boosted the depolymerization reaction and promoted further degradation to form a considerable amount of low molecular weight products. Even though there is trace content of AAEMs presented in lignocellulose, the pyrolytic pathway of cellulose would be changed to the production of light oxygenates and gases rather than levoglucosan [22,23]. In terms of the levoglucosan yield, the inhibition effect of AAEMs following the trend: $K^+ > Na^+ > Ca^{2+} > Mg^{2+}$ [24]. Therefore, removal of AAEMs, particularly K, would improve the yield of levoglucosan.

Table 1

Elemental analysis of biomass.

Samples	Organic elements (wt.%)			AAEMs (ppm)				
	C	H	N	K	Na	Mg	Ca	Total
Un-pretreated	45.8	6.2	0.2	7957.5	180.2	1023.2	3827.2	12988.1
200-1	44.1	6.4	0.0	1032.0	166.6	1041.2	2746.9	4986.8
200-5	43.8	6.3	0.0	900.1	156.9	673.9	3125.5	4856.5
200-10	43.4	6.4	0.0	706.0	149.5	636.7	2738.0	4230.2
220-1	43.8	6.3	0.0	964.6	143.2	649.7	3285.6	5043.1
220-5	43.3	6.7	0.0	577.1	126.1	639.7	3162.4	4505.3
220-10	43.1	6.4	0.0	570.9	116.9	620.5	2663.0	3971.3
240-1	43.3	6.3	0.0	662.2	140.9	640.7	3313.7	4757.5
240-5	43.4	6.3	0.0	642.2	123.4	530.2	2999.1	4294.9
240-10	43.7	6.3	0.0	539.4	115.9	523.5	2602.2	3781.0

3.2. Composition analysis

As shown in Fig. 1, the cellulose, hemicellulose and lignin accounted for 32.2%, 28.0% and 24.2% in un-pretreated corncobs. It was demonstrated that microwave-assisted glycerol pretreatment had the capacity to selectively eliminate lignin while retaining cellulose. The effect was observed stronger and stronger as pretreatment severity increased. The sample with the highest cellulose content (55.4%) and the least lignin content (10.5%) was pretreated at 240 °C for 10 min, where more than 56.6% lignin was removed. According to the previous research, the accumulation of cellulose facilitated levoglucosan production in fast pyrolysis of lignocellulose. There was a negative interaction between lignin and cellulose in pyrolysis of lignocellulose and it would promote the cellulose to form low molecular compounds instead of levoglucosan during fast pyrolysis. The interaction between hemicellulose and cellulose was evaluated weaker than that in lignin-cellulose, but this existence also suppressed the formation of levoglucosan, as well as significantly promoted the production of compounds derived from hemicellulose [25]. However, microwave-assisted glycerol pretreatment had a notable impact on the alteration of the main lignocellulosic composition, which would lead to an improvement in the formation of levoglucosan by the subsequent fast pyrolysis. Lignin was also considered to play significant roles in limiting the rate and extent of enzymatic hydrolysis of lignocellulose. Lignin limited the enzymatic hydrolysis mainly from two aspects. Basically, lignin served as physical barriers which have the ability to protect the cellulose from enzymatic degradation. Furthermore, lignin had been found to adsorb enzyme irreversibly, thus preventing their behaviors on cellulose. Since that, removal of lignin led to the increase of specific area, and was assisted to add the accessibility of enzyme to substrate and increase the enzymatic digestibility [3].

3.3. Crystalline analysis

The XRD patterns and the crystallinity of biomass are presented in Fig. 2 and Table 2, respectively. Cellulose's assembled structure contained amorphous and crystalline regions. The effect of pretreatment on crystallinity was considered to be dependent on the properties (composition and structure) of the biomass as well as the pretreatment conditions (time, temperature, and liquid to solid ratio, etc.). After microwave-assisted glycerol pretreatment, the crystallinity of corncobs increased. The highest crystallinity (58.6%) was obtained from the sample of 220–10. Microwave-assisted glycerol pretreatment was able to remove amorphous lignin, resulting in an enrichment of crystalline cellulose. In the research of Hosoya and Sakaki [26], cellulose with higher crystallinity gave a higher yield of levoglucosan. Amorphous sample was easier to decompose to a liquid phase than crystalline cellulose [27]. Less levoglucosan was yielded from cellulose with lower crystallinity than high crystallinity sample [27]. It had demonstrated that the crystal allomorph and the crystallinity of cellulose could influence the yield of levoglucosan, which was mainly originated from crystalline cellulose [28]. Therefore, the increase of crystallinity facilitated the formation of levoglucosan. However, the crystalline region of cellulose was more recalcitrant to bio-degradation than the amorphous fractions. During enzymatic hydrolysis, amorphous cellulose was hydrolyzed preliminarily, followed by the hydrolysis of crystalline cellulose. Higher crystalline caused a lower hydrolysis rate of biomass, which might be explained as increase of crystallinity had a negative effect on enzymatic hydrolysis.

3.4. Thermal behavior analysis

The TG/DTG profiles and characteristic parameters are exhibited in Fig. 3 and Table 2. The initial degradation temperature of raw corncobs was 149.4 °C. The decomposition was observed to begin at higher temperatures (152.9–165.5 °C) for the microwave-assisted glycerol pretreated samples. Furthermore, the maximum decomposition rate (DTG_{max}) and corresponding temperature (T_{max}) of un-pretreated corncobs were 1.0%/°C and 313.1 °C, which were lower than pretreated samples (328.4–341.4 °C, 1.3–2.1%/°C). It implied that the thermal stabilities of pretreated corncobs were enhanced, which could be ascribed to the release of AAEMs and structural alteration by pretreatment. The presence of AAEMs would lower the initial temperature for decomposition, and favor the formation of light oxygenates. Kim et al [29] observed that samples with higher CrI had a higher initial thermal decomposition temperature, which was in line with the result of thermal behavior analysis. The crystalline structure could impact the activation energy and thermal stability in the degradation of biomass [30,31]. The residue of un-pretreated corncobs was 24.2%, which was much higher than that of pretreated corncobs. Removal of lignin and AAEMs might be responsible for this result. Pyrolysis of lignin yielded more char than cellulose and AAEMs remained as ash in the residue.

3.5. Fast pyrolysis

The effect of microwave-assisted glycerol pretreatment on the pyrolytic product distribution was evaluated. The compounds identified by GC/MS included acids, ketones, aldehydes, furans, phenols and anhydrosugars (Table 3). In contrast to un-pretreated sample, the pyrolytic products of pretreated corncobs were concentrated and the types of products declined obviously. As a major product from cellulose pyrolysis, the relative content of levoglucosan was obviously higher than other compounds. The yield of levoglucosan increased evidently after microwave-assisted glycerol pretreatment (Fig. 4). Moreover, there was a substantial increase in the yield of levoglucosan with a further increase in pretreatment temperature or time. The highest yield of levoglucosan (44.5%) was obtained from the corncobs pretreated at 240 °C for 10 min, approximately over five-fold than that from raw corncobs (6.9%). The relative content of 2,3-andydro-d-mannosan was also increased from 0.1 to 0.9% after pretreatment. These results indicated that microwave-assisted glycerol had a remarkable enhancement on the production of andro-sugars in the pyrolysis of biomass, which might be ascribed to the dissolution of lignin and the removal of ash, as well as the enrichment of crystalline cellulose. Different from levoglucosan, the relative content of acetic acid as the major organic acid in pyrolysis products, was decreased from 5.4 to 0.5% after the pretreatment. Despite of acetic acid as the main product of

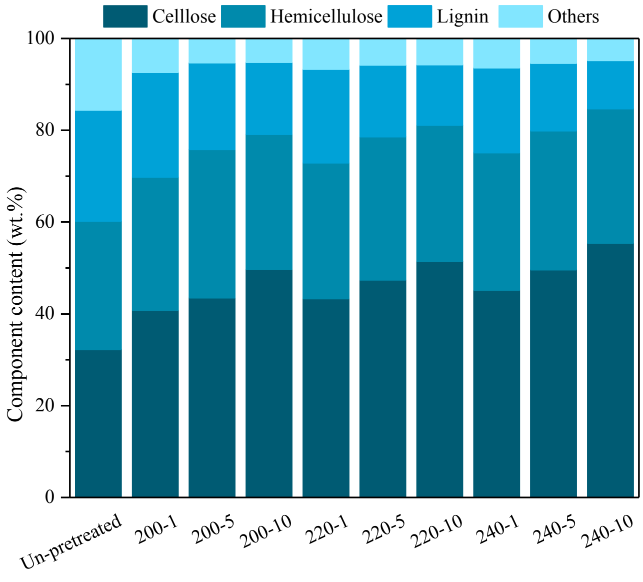

Fig. 1. The content of main components of un-pretreated and pretreated corncobs.

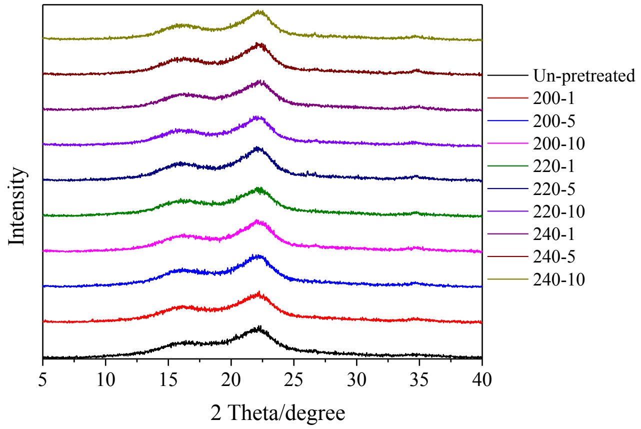

Fig. 2. XRD spectrograms of un-pretreated and pretreated corncobs.

Table 2
Characteristic parameters of biomass.

Samples	T_i (°C)	T_{max} (°C)	DTG_{max} (%/°C)	Residue (wt.%)	CrI (%)
Un-pretreated	149.4	313.1	1.0	24.2	46.7
200-1	157.2	340.6	1.3	13.3	49.3
200-5	154.9	340.9	1.4	11.1	52.2
200-10	159.9	338.7	1.4	12.9	52.3
220-1	157.8	341.4	1.3	13.4	55.9
220-5	160.4	332.5	1.6	13.0	56.6
220-10	157.0	332.2	1.8	8.2	58.6
240-1	152.9	340.7	1.4	11.1	54.9
240-5	165.5	330.9	1.7	9.3	56.9
240-10	157.4	328.4	2.1	12.0	55.8

T_i: The temperature of initial volatilization.
T_{max}: The temperature of the maximum mass loss rate.
DTG_{max}: The maximum decomposition rate.
Residue: The proportion of pyrolytic residue.

decarboxylation reaction of hemicellulose. Microwave-assisted glycerol pretreatment also resulted in a less amount of acetic acid in the pyrolysate of lignocellulose. The relative productions of phenols (such as phenol, 2,6-dimethozy-phenol, 2-methoxy-4-vinyl-phenol), which was generally regarded as the products of lignin pyrolysis, had a significant reduction after pretreatment and dropped with elevated temperatures or residence times in pretreatment. This phenomenon could be explained by the release of lignin during pretreatment.

3.6. *Enzymatic saccharification*

The concentration and yield of glucose contained in the enzymatic hydrolysate are given in Table 4 and Fig. 5. The delignification during microwave-assisted glycerol pretreatment was beneficial to increasing the susceptibility of biomass to the enzyme and thus promoted enzymatic saccharification. In enzymatic hydrolysis, the corncobs after pretreatment exhibited higher cellulose digestibility. The highest concentration of glucose was reached to 3.1 g/L as compared with 0.7 g/L for un-pretreated material after 24 h hydrolysis. It was also demonstrated that microwave-assisted glycerol pretreated corncobs gave glucose yields of 45.5–52.1%, which were obviously higher than that of un-treated corncobs (19.9%).

Compared with fast pyrolysis, obtaining fermentable sugars by enzymatic hydrolysis had theoretically superior performances, such as moderate operation condition, excellent selectivity and no microbial inhibitors preserved in the hydrolysate. In spite of the promise of enzymatic hydrolysis, high costs of cellulase, low concentrations of glucose, and slow saccharification rates restricted its industrial applications. The glucose yield could reach as high as 52.1%, but the highest concentration of glucose was only 3.1 g/L. However, in the process of fast pyrolysis, fast rates of saccharification (second level), high concentrations of levoglucosan, and the economic viabilities were the prominent advantages of fast pyrolysis. On the pyrolysate, the maximum yield and content of levoglucosan from cellulose could achieve 70.1 wt.% and 80%, respectively [32]. High content of feedstock for

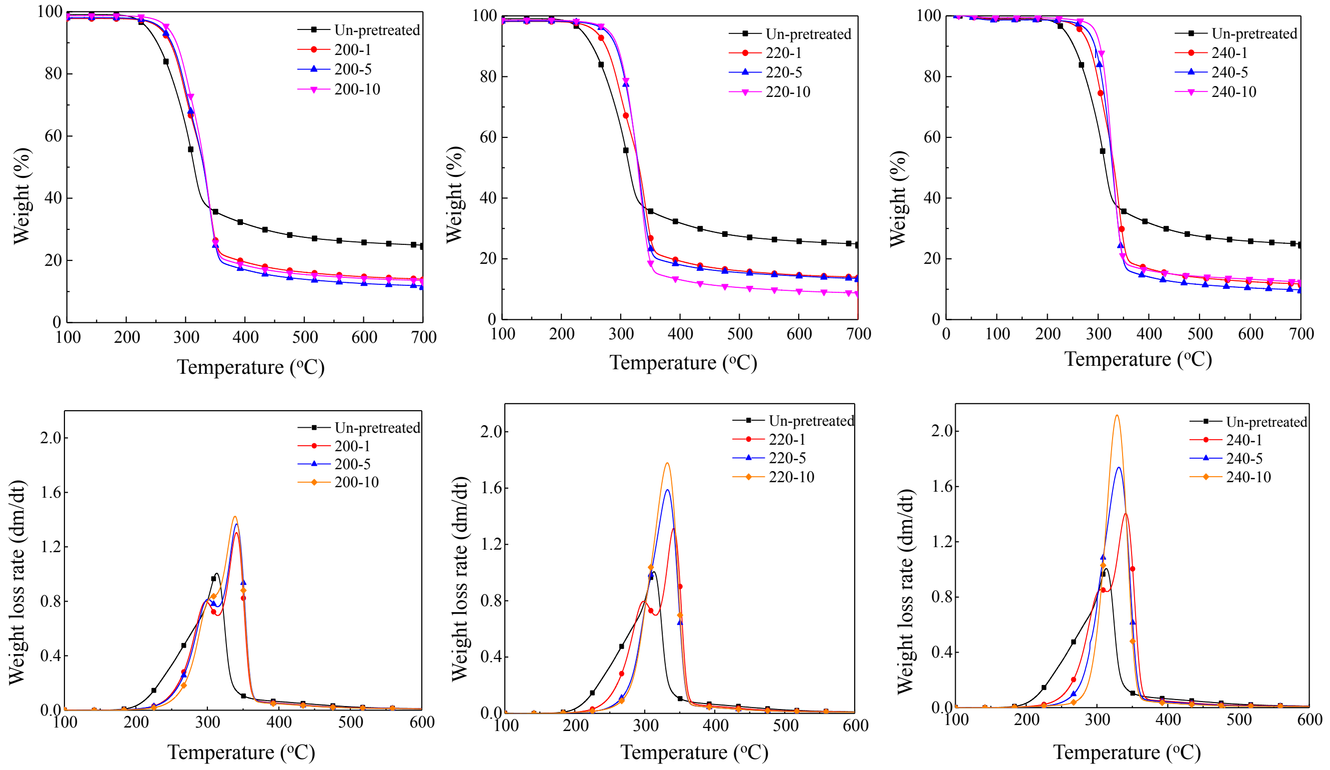

Fig. 3. TG and DTG curves of un-pretreated and pretreated corncobs.

Table 3
Relative contents of main compounds of fast pyrolysis of biomass.

Time	Compounds	Un-pretreated	200-1	200-5	200-10	220-1	220-5	220-10	240-1	240-5	240-10
4.4	Formaldehyde	0.6	2.8	3.2	3.1	2.1	3.2	3.2	3.6	3.2	1.3
4.7	Acetaldehyde	0.9	0.8	0.8	0.6	0.5	0.7	0.7	0.8	0.6	0.3
5.2	Furan	0.1	0.2	0.2	0.2	0.1	0.1	0.2	0.1	0.2	0.1
5.6	Acetone	0.5	0.2	0.1	0.1	0.1	0.1	0	0.1	0.1	0
6.4	2-Methyl-furan	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0
7.1	2,3-Butanedione	1.1	1.0	0.9	0.6	0.8	0.7	0.3	0.8	0.6	0.6
8.1	Hydroxy-acetaldehyde,	4.8	7.8	9.2	10.2	8.0	7.3	7.5	8.6	7.6	6.7
9.2	Acetic acid	5.4	3.1	1.8	1.1	2.0	1.3	0.8	1.8	0.7	0.5
12.3	Propanoic acid	0.3	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.2	0.1
13.6	1-Hydroxy-2-butanone	0.7	0.4	0.3	0.1	0.2	0.1	0.1	0.1	0.1	0.1
15.7	Furfural	1.5	1.9	1.9	1.9	2.3	1.9	1.8	2.2	2.1	1.8
17.1	2-Furanmethanol	1.0	0.6	0.5	0.5	0.6	0.2	0.2	0.3	0.3	0.3
17.4	1-(Acetyloxy)-2-propanone	0.5	0.4	0.3	0.2	0.4	0.3	0.3	0.3	0.3	0.2
19.3	1,2-Cyclopentanedione	1.6	1.9	1.7	1.4	1.6	1.2	1.2	1.4	1.1	1.0
21.2	2(5H)-Furanone	0.6	0.9	0.9	0.7	0.8	0.5	0.5	0.7	0.5	0.4
21.6	5-Methyl-2(5H)-furanone	0.1	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.1	0.1
23.3	Phenol	0.8	0.3	0.2	0.1	0.3	0.2	0.1	0.2	0.1	0.1
27.8	2,3-Anhydro-d-mannosan	0.1	0.7	0.4	0.4	0.4	0.5	0.6	0.9	0.5	0.8
29.4	3-Methyl-2,4(3H,5H)-furandione	0.6	0.5	1.3	1.0	0.7	0.9	1.0	1.0	0.4	1.0
30.1	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	0.2	0.3	0.3	0.3	0.3	0.4	0.3	0.1	0.3	0.2
30.5	2,3-Dihydro-benzofuran	5.5	2.6	2.1	1.3	2.8	1.4	1.2	2.1	0.7	0.2
30.7	2-Methoxy-4-vinyl-phenol	4.2	2.7	1.9	1.3	2.8	1.2	1.1	1.9	0.9	0.4
31.6	5-Hydroxymethylfurfural	0.3	0.8	0.9	1.2	0.8	1.1	1.1	1.0	1.3	1.2
32.0	2,6-Dimethoxy-phenol	1.4	0.6	0.5	0.3	0.6	0.4	0.3	0.4	0.1	0.1
34.9	3-Hydroxy-benzaldehyde	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.2	0.1	0.1
39.9	Levoglucosan	6.1	10.2	15	20.7	11.4	24.4	27.3	15.9	31.1	34.7

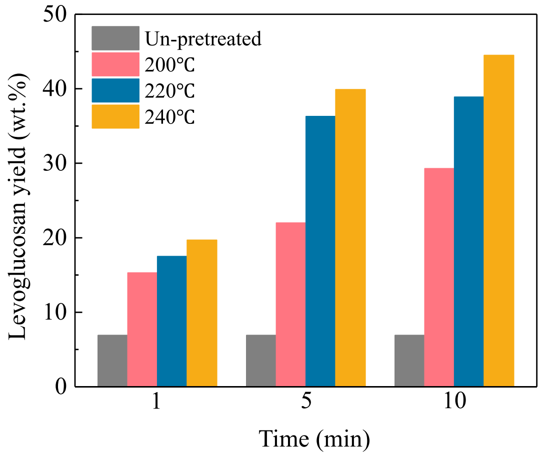

Fig. 4. Levoglucosan yield of fast pyrolysis of biomass.

Table 4
The concentration of glucose in enzymatic hydrolysate.

Samples	Glucose concentration (g/L)				
	2 h	4 h	8 h	12 h	24 h
Un-pretreated	0.2	0.3	0.4	0.5	0.7
200-1	0.5	0.8	1.2	1.5	2.1
200-5	0.6	0.9	1.4	1.7	2.3
200-10	0.7	1.2	1.6	2.0	2.7
220-1	0.6	0.9	1.4	1.7	2.3
220-5	0.7	1.2	1.6	2.0	2.6
220-10	0.8	1.4	1.9	2.3	2.9
240-1	0.8	1.1	1.6	2.0	2.6
240-5	0.9	1.3	1.9	2.3	2.9
240-10	0.9	1.4	2.0	2.4	3.1

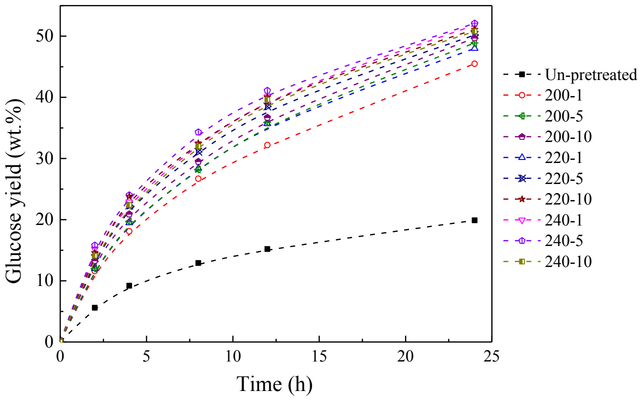

Fig. 5. Glucose yield of enzymatic hydrolysis of biomass.

fermentation favored the accumulation of fermentation products of high concentration and thereby saving the cost of product separation. The techno-economic comparison had demonstrated that the fast pyrolysis pathway had lower capital cost and operation fees than these of enzyme hydrolysis process [33,34]. However, the pyrolysate of lignocellulose contained a noticeable amount of toxic compounds (*e.g.,* acetic acid, phenols), which would suppress the microbial growth and productivity. Microwave-assisted glycerol pretreatment, which could not only improve the yield of levoglucosan but also decrease the formation of microbial inhibitors, was considered as a vital way to promote the fermentable application of pyrolytic sugars.

4. Conclusions

In this study, the effect of microwave-assisted glycerol pretreatment prior to fast pyrolysis and enzymatic saccharification for fermentable sugars production from corncobs was investigated. In fast pyrolysis, due to demineralization, delignification and concentration of crystalline cellulose through pretreatment, the yield of levoglucosan was improved dramatically (from 6.9 to 44.5%). Meanwhile, lignin removal for pretreated corncobs also caused a considerable elevation in glucose yield (from 19.9 to 52.1%) in enzymatic hydrolysis. Conclusively, microwave-assisted glycerol pretreatment was suggested to be considered for further development to obtain fermentable sugars from lignocellulose either by fast pyrolysis or enzymatic saccharification.

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LQJ and YXW designed the experiments, conceived of the study, performed the statistics analysis and draft manuscript. NNW, HQZ, YCZ and FZ participated made contributions to acquisition of data and statistical analysis. ZLZ and ZF participated in the design of the study. ZLZ, HBL and ZF also help to revise the manuscript. All authors read and approved the final manuscript.

Declaration of interest statement

The manuscript entitled "**Selective saccharification of microwave-assisted glycerol pretreated corncobs via fast pyrolysis and enzymatic hydrolysis**" with authors Li-qun Jiang, Ya-xiang Wu, Nan-nan Wu, Hui-qiong Zhong, Ying-chuan Zhang, Zeng-li Zhao, Hai-bin Li, Fan Zhang is the original work of the authors. We confirm that this manuscript has not been published elsewhere in whole or in part and is not under consideration by another journal. Approvals of all of the Authors' institutions have been granted to publish this work. All authors have approved the manuscript and agree with submission to *Fuel.* The authors have no conflicts of interest to declare.

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